PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

ANTHONY J. KINNEY ET AL.

CASE NO.: BB1538USNA APPLICATION NO.: 10/776311

CONFIRMATION NO.: 4023 GROUP ART UNIT: 1638

EXAMINER: DAVID T. FOX FILED: FEBRUARY 11, 2004

FOR: PRODUCTION OF VERY LONG CHAIN POLYUNSATURATED FATTY ACIDS IN OIL SEED PLANTS

Via EFS-Web

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Request for Rehearing of the Decision on Appeal dated November 1, 2010

Appeal 2009-015186

Application 10/776,311

Technology Center 1600

This is a request for rehearing of the Decision on Appeal of Appeal 2009-015186 dated November 1, 2010 pursuant to 37 C.F.R. §41.52. This request for rehearing is being submitted within two months of the date of the original November 1, 2010 decision of the Board. It is respectfully submitted that the Board has misapprehended or overlooked certain points as is set forth herein below.

REMARKS

1) If Prior Art is not available under 35 U.S.C. §102, then it is not available under 35 U.S.C. §103.

As was discussed in both the Brief on Appeal (page 9) and in the Reply Brief (page 5), Application 10/776,311 was filed non-provisionally on February 11, 2004 and claimed the priority benefit of Application 60/446,941 filed on February 12, 2003. Thus, Application 10/776,311 is entitled to a priority date of February 12, 2003.

All of the data and examples presented in Application 10/776,311 were present in Application 60/446,941 filed on February 12, 2003. Thus, Application 60/446,941 filed on February 12, 2003 demonstrated in Tables 7 and 8:

> A transgenic oilseed plant that produces mature seeds in which the total seed fatty acid profile comprises at least 1.0% of omega-3 polyunsaturated fatty acid(s) having at least twenty carbon atoms and five or more carbon-carbon double bonds, wherein the transgenic oilseed plant comprises in its genome at least two transgenic nucleic acid sequences encoding at least two different polypeptides, and further wherein at least one polypeptide has desaturase activity and at least one polypeptide has elongase activity.

It is stated in MPEP §2141.01 that subject matter that is prior art available under 35 U.S.C. §102 is available under 35 U.S.C. §103.

It is stated in MPEP §2128.02 that a magazine or technical journal is effective as of its date of publication.

Finding of Fact 24 in the Decision on Appeal in references Robert, "Production of Eicosapentaenoic and Docosahexaenoic Acid-Containing Oils in Transgenic Land Plants for Human and Aquaculture Nutrition", *Marine Biotechnology*, 8:103-109 (2006), as disclosing Abbadi et al. (2004).

The citation for the Abbadi et al. article mentioned by Robert is the following: Abbadi et al., *The Plant Cell*,. 16:2734-2748 (October, 2004). A copy

of Abbadi et al., as referenced by Robert, is provided herewith for the convenience of the Board in order to confirm the actual publication date.

Both Robert and Abbadi et al. **postdate** the February 11, 2004 filing date and the February 12, 2003 priority date to which Application 10/776,311 is entitled.

If neither the Robert nor Abbadi et al. reference is available as prior art under 35 U.S.C. §102, then it cannot be available for use as a reference under 35 U.S.C. §103.

2. The state of the art at the time that Application 10/776,311was filed in February, 2003 was based on data obtained from: (i) transgenic yeast fed an exogenous substrate and (ii) one transgenic plant producing GLA (18:3, omega-6).

A. It should be noted that all of the data provided in Application 10/776,311, having a priority date of February 12, 2003, was obtained from transgenic oilseed plants, and their seeds, capable of making all of the *in vivo* substrates required for the production of an omega-3 polyunsaturated fatty acid ["PUFA"] having at least twenty carbons and further having 5 or more double bonds.

B. For convenience, a chart is set forth herein setting forth nomenclature regarding some relevant PUFAs to the discussion below. Omega-3 polyunsaturated fatty acids ["PUFAs"] having at least twenty carbons and further having 5 or more double bonds, as claimed in 10/776,311, are highlighted by shading.

Nomenclature of Polyunsaturated Fatty Acids And Precursors

Common Name	Abbreviation	Chemical Name	Shorthand Notation
Oleic	--	*cis*-9-octadecenoic	18:1
Linoleic	LA	*cis*-9, 12-octadecadienoic	18:2 ω-6
γ–Linolenic	GLA	*cis*-6, 9, 12-octadecatrienoic	18:3 ω-6
Stearidonic acid	SDA	cis-octadeca-3,6,9,12-tetraenoic acid	18:4 ω-3
Dihomo-γ–Linolenic	DGLA	*cis*-8, 11, 14- eicosatrienoic	20:3 ω-6
Arachidonic	ARA	*cis*-5, 8, 11, 14-eicosatetraenoic	20:4 ω-6
Eicosa-pentaenoic	EPA	*cis*-5, 8, 11, 14, 17-eicosapentaenoic	20:5 ω-3
Adrenic Acid	ADA	*cis*-7,10,13,16-docosatetraenoic	22:4 ω-6
Docosa-pentaenoic	DPA	*cis*-7, 10, 13, 16, 19-docosapentaenoic	22:5 ω-3
Docosa-hexaenoic	DHA	*cis*-4, 7, 10, 13, 16, 19-docosahexaenoic	22:6 ω-3

C. Knutzon et al. (U.S. Patent 6,075,183), issued June 13, 2000.

1. Knutzon et al. did **not** disclose a gene encoding an elongase required to make an omega-3 polyunsaturated fatty acid ["PUFA"] having at least twenty carbons and further having 5 or more double bonds.

2. Example 6 of Knutzon et al. references expression in yeast. Examples 7 and 8 of Knutzon et al. concern expression in an oilseed plant, i.e., *Brassica.* However, the transgenic oilseed plant made by Knutzon et al. expressed **either** a delta-6 desaturase **or** a delta-5 desaturase (but no elongase). Knutzon et al. did **not** make a transgenic oilseed plant co-expressing both a delta-6 desaturase and a delta-5 desaturase. It should be noted that even if a transgenic plant **was** produced co-expressing **both** a delta-6 and a delta-5 desaturase, such a plant would be **incapable** of producing an omega-3 polyunsaturated fatty acid ["PUFA"] having at least twenty carbons and further having 5 or more double bonds.

3. **Plants do not possess an endogenous elongase capable of making twenty carbon PUFAs with 5 or more double bonds.**

4. Example 7 of Knutzon et al. showed that leaves of a transgenic oilseed plant could produce arachidonic acid (ARA; 20:4, omega-6) from the substrate dihomo-gamma-linolenic acid (DGLA; 20:3, omega-6) via expression of a delta-5 desaturase. However, the DGLA substrate was supplied **exogenously** (Knutzon et al., col. 19 at line 16).

5. Example 8 of Knutzon et al. showed that seeds of a transgenic oilseed plant could produce gamma-linolenic acid (GLA; 18:3, omega-6) from the substrate linolenic acid (LA; 18:2, omega-6) via expression of a delta-6 desaturase. The GLA was made *in vivo* (without exogenously adding substrate). This appears to be the only example in Knutzon et al. in which the substrate was not provided exogenously. However, GLA is not an omega-3 PUFA and is not relevant to Claim 1 of Application 10/776,311.

D. Abbott Laboratories (WO 02/08401 A2), published Jan. 31, 2002 ("Abbott")

1. Finding of Fact 17 in the Decision on Appeal overlooks several important facts as stated on page 7 at lines 1-14 of Abbott:

a) Abbott never made a transgenic oilseed plant expressing **both** a desaturase and an elongase.

b) Abbott only made a transgenic yeast co-expressing a delta-5 desaturase and an elongase, wherein the transgenic yeast was **exogenously** provided substrate as discussed below.

c) The products mentioned on page 7 at lines 6-8 were dihomo-gamma-linolenic acid (DGLA; 20:3, omega-6) and adrenic acid (ADA; 22:4, omega-6). **Neither** of these products is an omega-3 PUFA having at least 20 carbon atoms and 5 or more double bonds and thus are not relevant to Claim 1 of Application 10/776,311.

2. Finding of Fact 18 in the Decision on Appeal overlooks the fact that "**exposing**" a substrate to an enzyme may involve exogenously adding the substrate as was the case in Example 3.

a) Pages 71-73 of Example 3 discuss the transformation and co-expression of the *M. alpina* elongase cDNA and a delta-5 desaturase in Baker's yeast grown in selective media, wherein the substrate gamma-linolenic acid (GLA; 18:3, omega-6) was **exogenously** provided to the yeast. The elongase was able to produce dihomo- gamma-linolenic acid (DGLA; 20:3, omega-6) from GLA; the delta-5 desaturase then desaturated DGLA to produce arachidonic acid (ARA; 20:4, omega-6).

b) At the end of Example 3 (page 79), it was postulated that the expression of delta-5, delta-6 and delta-12 desaturases, in yeast, along with the elongase should result in the production of arachidonic acid (ARA; 20:4, omega-6) without the need for an exogenous supply of fatty acids. However, the actual experiment was not performed. No transgenic yeast was made co-expressing an elongase, a delta-5 desaturase, a delta-6 desaturase and a delta-12 desaturase.

3. Abbott never produced an omega-3 fatty acid having at least twenty carbon atoms and having 5 or more double bonds *in vivo* in transgenic yeast. Neither was this accomplished in a transgenic oilseed plant.

E. Browse et al. (U.S. 6,884,921 B2), issued April 26, 2005.

1. Browse et al. disclosed only a **single** enzyme, i.e., an omega-3 fatty acid desaturase, and a single enzyme conversion.

2. Browse et al. showed in Example 4 that a transgenic Arabidopsis plant could produce docosahexaenoic acid (DHA; 22:6, omega-3) from the substrate docosapentaenoic acid (DPA; 22:5, omega-3) via expression of the omega-3 fatty acid desaturase, when DPA was supplied **exogenously (via spraying)**.

3. Finding of Fact 20 in the Decision on Appeal fails to appreciate that if exogenous application of the substrate DPA (via spraying) (Columns 19-20 and Examples 3 and 4) was required, because the Browse et al. transgenic plant could **not** make the substrate *in vivo*.

4. Browse et al. **never** made a transgenic oilseed plant co-expressing at least one desaturase and at least one elongase.

F. Exogenously providing substrate avoids a problem that *in vivo* production faces, i.e., in the shuttling of fatty acids between the phosphatidylcholine and coenzyme A pools.

1. Long-chain polyunsaturated fatty acid biosynthesis requires that all fatty acids are coupled either to:

a) phosphatidylcholine (PC) for any desaturation reaction; or,

b) coenzyme A (CoA) for any elongation reaction.

2. The "shuttling" of fatty acids from PC to CoA pools is achieved by acyl-transferases. The work of Abbadi et al., *The Plant Cell*, 16:2734-2748 (October, 2004) as discussed by Robert on page 105, showed that a fatty acid

coupled to PC is a substrate for delta-6 desaturase and the product of the desaturation is immediately channeled to triacylglycerols (i.e., effectively bypassing the acyl-CoA pool). This is illustrated in Figure 1 on page 104 of Robert, *Marine Biotechnology*, 8:103-109 (2006) (parenthetically, both Robert and Abbadi are not available as a reference; Section 1, *supra,* and Brief on Appeal, pages 10-11).

3. The substrate available for elongation is limited when the pool of delta-6 desaturated fatty acids coupled to CoA becomes low due to the channeling of the desaturated fatty acids to triacylglycerols . This happens because elongation reactions only occur when fatty acids are coupled to CoA.

4. If the delta-6-desaturated acyl-CoA substrate is limited, then the synthesis of elongated fatty acids (i.e., C20) will be limited. Thus, production of PUFAs having at least twenty carbon atoms and five or more carbon-carbon double bonds will be limited, since biosynthesis of these PUFAs requires at least one elongation reaction in combination with various desaturation reactions when the initial substrate is oleic acid (C18:1).

5. Exogenously providing delta-6-desaturated fatty acid substrate is deceptive because it bypasses the requirement for transfer of delta-6-desaturated fatty acids from the PC pool (where they are made *in vivo*) to the acyl-CoA pool. Bypassing this step artificially inflates the amount of delta-6-desaturated acyl-CoA in the acyl-CoA pool, compared to levels if the substrate was produced *in vivo*. As described in Abbadi et al. and Robert, when delta-6-desaturated fatty acid substrate is made *in vivo* in the PC pool, channeling directly to triacylglycerols occurs. This depletes the pool of substrate available for transfer to the acyl-CoA pool and subsequent elongation, as discussed above.

G. 15.67% of gamma-linolenic acid (18:3, omega-6) does not constitute at least 1.0% of omega-3 polyunsaturated fatty acid(s) having at least twenty carbon atoms and five or more carbon-carbon double bonds.

1. Knutzon et al. disclosed in Table 5 that a transgenic Brassica plant (expressing a delta-6 desaturase under the control of a seed-specific napin promoter) was able to produce 15.67 % GLA (18:3, omega-6) and 0.53% stearidonic acid (SDA, 18:4, omega-3). GLA is not an omega-3 fatty acid and SDA is an omega-3 fatty acid having eighteen carbon atoms and four carbon-carbon double bonds. Neither of these polyunsaturated fatty acids falls within the scope of the claimed invention. Parenthetically, it should be noted that 15.67% GLA represents only a 53% conversion of the substrate linoleic acid to GLA.

2. Assuming *arguendo* that 100% of the SDA could be shuttled to the acyl-CoA pool AND 100% could be elongated to ETA and 100% could be moved back to the PC pool AND 100% could be desaturated to make EPA and 100% could be transferred to the triacylglycerol pool (seed oil), this would result in only 0.53% EPA. Realistically, it is highly unlikely that each step in the process would function at 100% efficiency which means that the actual amount made would be less than 0.53%.

H. Conclusion

Accordingly, in view of the foregoing discussion, the cited references do not provide a reasonable expectation of success to one of ordinary skill in the art to make a transgenic oilseed plant that produces mature seeds in which the total seed fatty acid profile comprises at least 1.0% of omega-3 PUFA(s) having at least twenty carbon atoms and five or more carbon-carbon double bonds, wherein the transgenic oilseed plant comprises in its genome at least two transgenic nucleic acid sequences encoding at least one polypeptide having desaturase activity and at least one polypeptide having elongase activity.

Yeast that are fed exogenous substrate(s) do not render obvious a transgenic oilseed plant capable of making all of the substrates, *in vivo*, required for the production of at least 1.0% of omega-3 PUFA(s) having at least twenty carbons and further having 5 or more double bonds.

Respectfully submitted,

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